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Historical Prevalence of the Amphibian Chytrid Fungus (*Batrachochytrium dendrobatidis*) in West Africa

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ABSTRACT: The chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) is implicated in global declines and extinctions of amphibian species. Although *Bd* is found on all continents where amphibians persist, disease dynamics and prevalence remain poorly quantified and understood in certain regions of the world, such as West Africa. West Africa is characterized as a “safe haven” for amphibians despite niche models indicating that there is suitable habitat for *Bd* in this region. Therefore, a major knowledge gap remains regarding the threats that *Bd* poses to West African amphibians. We screened 3093 fluid-preserved amphibian specimens to determine the historical prevalence of *Bd* in West Africa. We detected five *Bd*-positive samples originating from Ghana and Benin, with the earliest positive occurrence dating back to 1969, suggesting that *Bd* has been present in West Africa for at least 54 yr. We obtained sequence data for two of these samples and both correspond to the *Bd*-GPL2 clade. West Africa hosts amphibian assemblages that are distinct from any other African region; thus continued *Bd* surveys are crucial for defining the extent of *Bd* across Africa and understanding whether this pathogen poses a threat to the unique amphibian diversity of this region.

Key words: Biodiversity; Conservation; Chytridiomycosis; Disease ecology

THE CHYTRID fungus *Batrachochytrium dendrobatidis* (*Bd*) is linked to global population die-offs and extinctions in amphibians (Berger et al. 1998; Wake and Vredenburg 2008; Scheele et al. 2019), resulting in the decline of >500 amphibian species, which is the greatest loss of vertebrate biodiversity attributed to a pathogen (Scheele et al. 2019). Although *Bd* is documented on all continents on which amphibians are found, *Bd*-associated declines are typically centered in tropical, montane forest regions where cool and moist climates support *Bd* growth (Berger et al. 1998; Lips et al. 2006; Olson et al. 2013, 2021; Lips 2016; Byrne et al. 2019; Scheele et al. 2019). Pathogen transmission is facilitated by several pathways in the environment, including direct contact with infected amphibian hosts and indirect contact with contaminated aquatic reservoirs such as streams, ponds, rainwater, and fog water or with contaminated vegetation and substrates (Berger et al. 1998; Rachowicz and Vredenburg 2004; Kolby et al. 2015a,b; Prado et al. 2023). Infection causes the disease chytridiomycosis, which disrupts vital skin functions such as respiration, electrolyte transport, and osmotic balance, typically leading to mortality (Voyles et al. 2009; Van Rooij et al. 2015). However, amphibian host responses to chytrid infection vary widely across taxa. Some species sustain low infection levels, thus operating as carriers of the fungus (*Lithobates cat- esbianus*, Daszak et al. 2004; *Xenopus laevis*, Weldon et al. 2004), whereas others are highly susceptible and experience lethal effects (*Taudactylus acutirostris*; Schloegel et al. 2006; Van Rooij et al. 2015). These varied responses to *Bd* infection demonstrate the complexity of the ecological factors influencing

the outcome of *Bd*–amphibian interactions. Although the mechanism of *Bd* infection is well studied, major knowledge gaps remain in our understanding of pathogen–host dynamics, including in terms of host identity, pathogen strain, and the pathogen’s global extent.

Although *Bd*-associated declines are well documented in some regions (e.g., Brazil, Panamá, Mexico, Australia, and the Caribbean Islands; Lips et al. 2006; Scheele et al. 2019), the extent of declines remains unclear in many parts of the world, including across much of Africa. To date, published data include 16,920 *Bd* sampling records (presence and absence) in Africa (Ghose et al. 2023), with most studies centered in eastern Africa (Burundi, Ethiopia, Kenya, Malawi, Mozambique, Rwanda, Tanzania, Uganda, and Zambia), southern Africa (Botswana, Lesotho, Namibia, South Africa, and Swaziland), and Central Africa (Cameroon, Central African Republic, Democratic Republic of Congo, Equatorial Guinea, Gabon, São Tomé and Príncipe). In North Africa, Morocco is the only country that has been surveyed for *Bd* (El Mouden et al. 2011; Ait El Cadi et al. 2019; Thumsová et al. 2022) and one study to date has been conducted in West Africa, representing a major geographic sampling bias across the continent.

West Africa remains one of the most undersurveyed regions for *Bd* in Africa, especially considering the diverse and unique amphibian fauna in this global biodiversity hot spot (Myers et al. 2000). The only study investigating *Bd* prevalence in this region included 793 samples comprising 62 amphibian species collected opportunistically between 1993 and 2011 from 7 of 17 West African countries (Benin, Burkina Faso, Côte d’Ivoire, Ghana, Guinea, Liberia, and Sierra Leone; Penner et al. 2013). This survey did not find evidence of *Bd* in amphibian populations in West Africa west of the Dahomey Gap—a naturally nonforested corridor stretching from eastern Ghana to western

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Nigeria. Yet, multiple studies have reported high *Bd* prevalence across the rest of the African continent, including the Central African country of Cameroon (Soto-Azat et al. 2010; Baláz et al. 2012; Zimkus et al. 2020; Ghose et al. 2023). Despite this, there are no reports of *Bd* in neighboring West African countries. Additionally, environmental niche modeling—which predicts suitable habitat for a species using associations between known occurrence data and environmental conditions—indicates suitable habitat for *Bd* in West Africa, given the wet and tropical conditions of the Upper Guinean Forest system (Penner et al. 2013; Zimkus et al. 2020). This western African tropical forest network possesses the highest risk for *Bd* invasion on continental Africa (Ghose et al. 2023), which is particularly concerning because West Africa's amphibian assemblages—comprising over 170 species—are distinct from Africa's six other biogeographic regions (Penner et al. 2011). The total number of West African amphibians screened to date is remarkably low given the large geographic area (8 million km² across 17 countries) and species diversity, which underscores a need to extensively survey amphibian communities in West Africa.

A better sense of *Bd* disease dynamics requires understanding not only the environmental variables that influence its distribution but also the various factors that explain host susceptibility. Susceptibility to *Bd* varies among and within species and with life history (e.g., aquatic breeders or direct-developing species), habitat, and climate (Scheele et al. 2019). For instance, species-decline severity is greatest in consistently wet regions, at high elevations (Lips et al. 2003; Stuart et al. 2004; Kielgast et al. 2010), and in species associated with perennial aquatic habitats (i.e., aquatic breeders, particularly stream-dwelling amphibians; Scheele et al. 2019). The intensity of infection is also dependent on the *Bd* strain, of which there are several that vary in pathogenicity and distribution. Globally, five lineages of *Bd* are known: (1) the highly virulent “global panzootic lineage” *Bd*GPL (including two clades: *Bd*GPL-1 and *Bd*GPL-2); (2) *Bd*CAPE, which was first identified in Cape Province, South Africa; (3) *Bd*Brazil/ASIA2 described from samples in South Korea and Brazil; (4) *Bd*ASIA1 described from samples collected in South Korea and Switzerland; and (5) *Bd*ASIA3 described from samples collected in Indonesia, the Philippines, and China (O'Hanlon et al. 2018). In Africa, two strains of *Bd* are known to infect amphibian hosts in the wild: *Bd*GPL and the less virulent *Bd*CAPE. Although the genotypes of *Bd* that may exist in West Africa are unknown, the dominant strain in Cameroon—a neighboring Central African country—is *Bd*CAPE (O'Hanlon et al. 2018). Identifying the *Bd* lineage provides insight into its evolution and origin (Hydeman et al. 2017) and could reveal mechanisms of spread (e.g., if *Bd* was anthropogenically introduced or is endemic in amphibian populations) and diversification (Rodríguez et al. 2014).

The geographic extent of *Bd* in West Africa and the strains of *Bd* that may be present there are critical knowledge gaps threatening amphibian diversity and impeding proactive conservation measures (Penner et al. 2013). Pairing amphibian surveys with *Bd*-genotyping methods are the mechanisms by which one can monitor and understand the pathogen's prevalence, spread, and evolution. In this study, we leverage museum specimens to determine the historical prevalence of *Bd* in West Africa, show that *Bd* is likely to be detected with increased sampling effort, and characterize

Bd strains present in West Africa. Our study provides novel insights into the historical distribution of *Bd* in West Africa and the potential dynamics of *Bd* invasion in this region.

MATERIALS AND METHODS

Museum Sampling

To conduct a comprehensive survey of historical *Bd* prevalence in West Africa, we screened 3093 amphibians from museum collections housed at the California Academy of Sciences (CAS: $n = 1773$) and University of California, Berkeley Museum of Vertebrate Zoology (MVZ: $n = 1320$), which represents roughly 34% of all West African amphibian specimens ($n = \sim 9221$) in databased collections (Supplemental Material S1, S2, available online). These collections are comprised of 11 anuran families (Ranidae, Arthroleptidae, Pipidae, Hyperoliidae, Bufonidae, Rhacophoridae, Microhylidae, Hemisotidae, Ptychadenidae, Conrauidae, and Petropedetidae) and two caecilian families (Dermophiidae and Caeciliidae) encompassing a wide range of life histories that may vary in their susceptibility to *Bd* and span almost a century (1921–2006) of biological field surveys in 13 West African countries (Sierra Leone, Côte d'Ivoire, Ghana, Guinea, Nigeria, Niger, Liberia, Burkina Faso, Togo, Benin, Senegal, Mali, and Mauritania). Sampled specimens included both aquatic breeders and direct-developing species. Specimen swabbing took place from 1 June to 10 July 2022 at the CAS and from 11 to 18 July 2022 at the MVZ. Following a swabbing protocol described by Hyatt et al. (2007), we rinsed each specimen with 70% ethanol to prevent cross-contamination before swabbing the ventral surface of the hind limbs, thighs, digits, and pelvis using a sterile cotton rayon swab (Medical Wire, Corsham, UK). Swabs were stored at room temperature in 1.5-mL microcentrifuge tubes containing 0.1 mL of 95% ethanol until they were processed for genetic data collection.

Bd Detection of Museum Samples

To extract deoxyribonucleic acid (DNA) from the *Bd* swabs, we first evaporated the 95% ethanol from the samples using an Eppendorf concentrator plus (Eppendorf, Hamburg, Germany). We then followed established methods for the extraction of *Bd* DNA for quantitative polymerase chain reaction (qPCR) using ABI Prepman Ultra (Boyle et al. 2004).

To conduct TaqMan qPCR assays, we amplified the internal transcribed spacer 1 (*ITS1*) gene using the primer pair ITS1-3 Chytr and 5.8S Chytr (Boyle et al. 2004). Each qPCR reaction was carried out in a 25- μ L volume at a 1:5 dilution containing 5 μ L of template DNA, 12.50 μ L of Sensi-Fast Lo-ROX master mix, 1.13 μ L of each primer, 1.2 μ L of Chytr MGB2, 1 μ L of bovine serum albumin, 0.83 μ L of 10 $\times$ exo-internal positive control (IPC) mix, 0.17 μ L of exo-IPC DNA, and 2.05 μ L of molecular-grade water. We pooled five samples into each qPCR well, an approach used when *Bd* prevalence is low (e.g., *Bd* swabs from museum samples), to reduce costs, especially when processing many samples. We ran each qPCR plate with standards that had a *Bd* concentration ranging from 0.1 to 1000 ITS copies (Gblocks synthetic standards, Integrated DNA Technologies) and two blanks containing Taqman master mix and molecular-grade water. Samples were then analyzed using

the Applied Biosystems QuantStudio 3 (Thermo Fisher Scientific, Waltham, MA). Pooled samples with positive reads were disaggregated and qPCR was repeated on these unpooled samples to determine the individuals that were *Bd* positive.

We ran a Clopper–Pearson test (exact binomial distribution confidence interval [CI] test) to determine the 95% CIs for *Bd* prevalence (Clopper and Pearson 1934; Bales et al. 2015; Fitzpatrick et al. 2018; Alabai et al. 2021). The Clopper–Pearson test calculates the lower and upper bound intervals of what true *Bd* prevalence could be in the population.

Bd Sequencing of Positive Samples and Phylogenetic Analysis

We sequenced *Bd*-positive samples by modifying a custom genotyping assay (Byrne et al. 2017) to optimize the protocol for degraded museum DNA. This assay targets 96 amplicons that are 150–200 base pairs (bp) long and are in phylogenetically informative regions of the *Bd* genome. First, we preamplified raw DNA extracts using droplet PCR to help counteract the effect of PCR inhibitors and increase the sensitivity of our PCR assay. For this step we made a master mix with the following components: 10.5 μ L of 2 $\times$ droplet digital PCR supermix for probes (no deoxyuridine triphosphate; Bio-Rad Laboratories, Inc.), 4.2 μ L of primer pool consisting of 96 forward and reverse primers at a concentration of 520 nM each, 1.05 μ L of PCR-grade water, and 5 μ L of the DNA extract. We then used the Bio-Rad QX200 droplet generator to partition our PCR into >10,000 droplets and ran PCR using the following thermocycling profile: 95°C for 10 min, 50 cycles of 94°C for 30 s and 60°C for 2 min (using a ramp rate of 1°C/s), 98°C for 10 min. We extracted the amplified products from the droplets using a chloroform extraction protocol (Bio-Rad Droplet Digital Applications Guide) and cleaned the extracted DNA

products using 2 μ L of EXO-SAPit (Applied Biosystems) to remove primers. Our cleaned, preamplified samples were then sequenced using a Fluidigm access array 48 $\times$ 48 integrated fluidic circuit (Standard BioTools, Inc.) at the University of Idaho IBEST Genomics Resources Core. This method uses microfluidics to amplify and barcode all 96 amplicons using 48 separate primer pools consisting of two primer pairs. Barcoded samples were then pooled and sequenced on one lane of an Illumina MiSeq using the 300-bp paired-end kit.

We preprocessed all raw sequences as described in Byrne et al. (2017). We filtered reads by selecting sequence variants that were present in at least five reads and represented at least 5% of the total number of reads for that sample/locus using dbcAmplicons (available at <https://github.com/mset/tles/dbcAmplicons>). We generated consensus sequences for all samples using International Union of Pure and Applied Chemistry (IUPAC) ambiguity codes for multiple alleles. We then used gene-tree to species-tree approach to construct a phylogeny for the target sequences that passed filtration (at least five amplicon sequences) and 33 reference samples of known *Bd* lineage (Byrne et al. 2019). First, we trimmed our consensus sequence data set to eliminate loci that had >50% missing data for the reference samples, resulting in 92 loci. Next, for each amplicon we individually aligned all sequences using the MUSCLE package (v3.4.3; Edgar 2004) in R, checked the alignments for errors in Geneious (v2023.2.1; Kearse et al. 2012), and used the RAxML plugin (Stamatakis 2014) in Geneious to search for the best-scoring maximum-likelihood tree for each locus using rapid bootstrapping (100 replicates). We then collapsed all branches in all amplicon trees with <10 bootstrap support and used Astral-III to generate a consensus tree (Zhang et al. 2018). Astral generates an unrooted species tree given a set of unrooted gene trees and is robust to missing data. We collapsed nodes within the Astral consensus

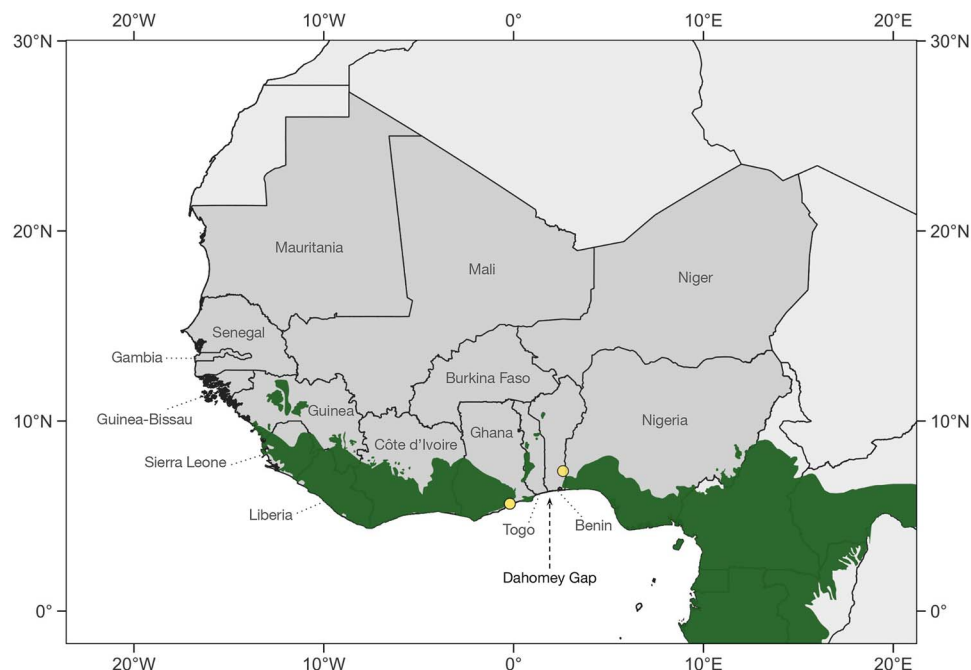


FIG. 1.—*Batrachochytrium dendrobatidis* (*Bd*) presence in West Africa. Positive records of *Bd* in Ghana and Benin are indicated by yellow circles. West African countries are shaded, and forested regions are shown in green.

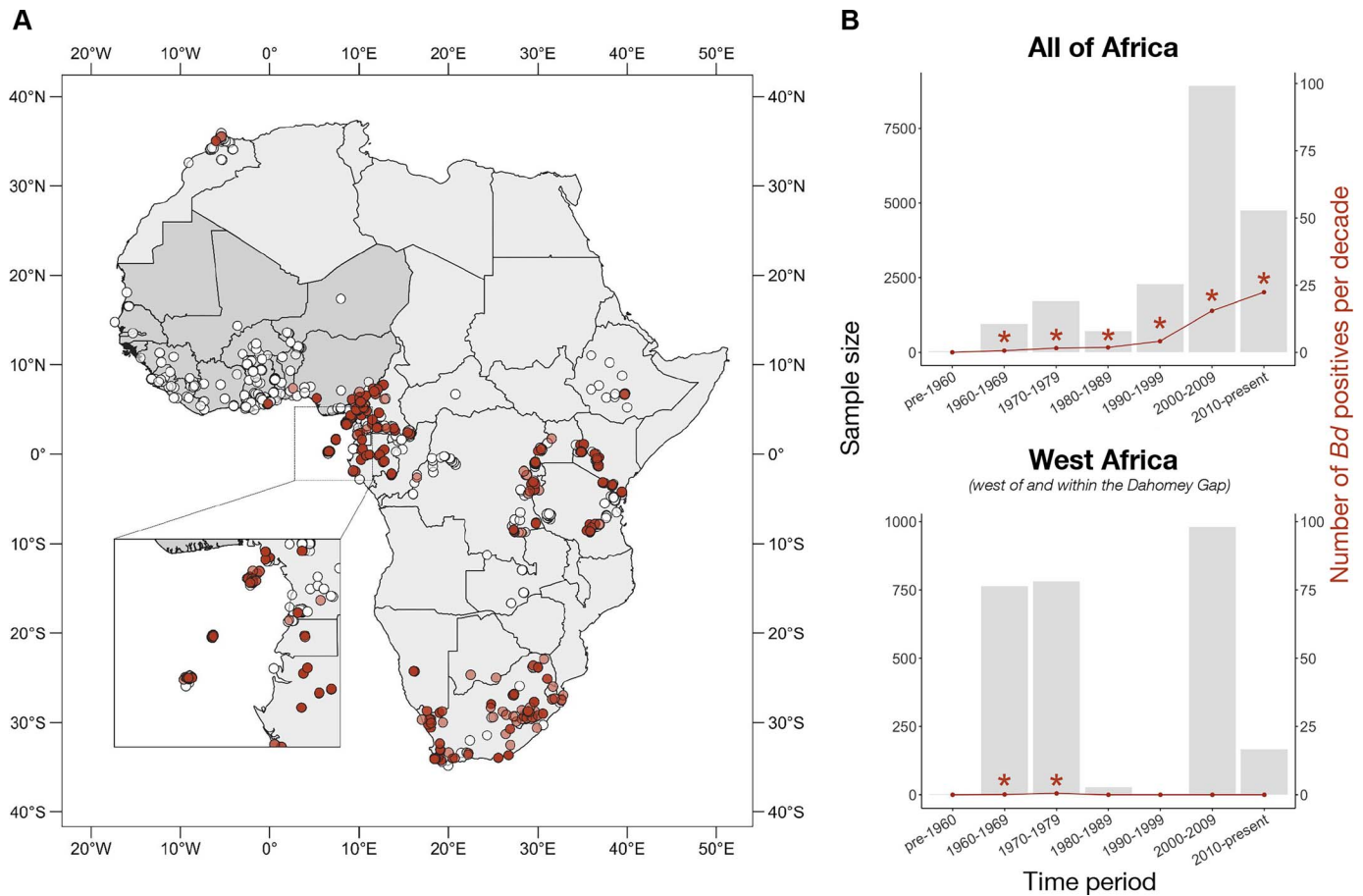


FIG. 2.—Spatial and temporal distribution of *Batrachochytrium dendrobatidis* (*Bd*) records in Africa that includes samples collected from museum specimens and from live animals in field settings. (A) All positive *Bd* (red circles) and negative *Bd* (white circles) occurrences in Africa to date that have georeferenced coordinates ($n = 12,954$). (B) Bar plots are the total number of *Bd* records (omitted 739 records that did not have data for year collected) by decade (pre-1960, 1960–1969, 1970–1979, 1980–1989, 1990–1999, 2000–2009, 2010–present) and red dots indicate the number that were *Bd* positive by decade in all of Africa (top panel) and in West Africa alone (samples west of and within the Dahomey Gap; bottom panel). Note that no specimens collected between 1990 and 1999 in West Africa were tested for *Bd*. Asterisks indicate decades that have *Bd*-positive occurrences.

tree with <0.5 posterior probability and rooted the resulting tree on the *Bd*-ASIA3 lineage (Byrne et al. 2019). Finally, since one museum sample only returned a single amplicon sequence, we analyzed this single amplicon sequence alignment to determine likely *Bd* lineage rather than including this sample in the phylogenetic analysis.

RESULTS

Historical *Bd*-Positive Occurrences in West Africa

We screened 3093 samples and detected 5 *Bd*-positive occurrences (Fig. 1), resulting in an overall *Bd* prevalence of 0.16% (95% CI: $0.0005 \leq 0.0016 \leq 0.0037$) for our West Africa samples. All 5 samples were collected from a single host species, *Kassina senegalensis*, with 4 samples collected from the Greater Accra Region in Ghana (5.647°N , 0.194°W ; outside Dahomey Gap) in 1979 (CAS 153500–153503) and 1 collected from the Plateau Department in Benin (7.365°N , 2.605°E ; within Dahomey Gap) in 1969 (CAS 165580).

We amassed 20,281 *Bd* positive/negative records from published data and this present study spanning 167 yr (1852–2019) of *Bd* surveys in Africa (Fig. 2A). This comprehensive data set includes samples collected from museum specimens and from live animals in field settings. Of these

records, 2589 (12.77%) represent positive records of *Bd*. We contributed new *Bd* survey data for five West African countries (Mali, Mauritania, Niger, Senegal, Togo) that have not been included in contemporary *Bd* surveys and note that there are temporal gaps in *Bd* sampling in West Africa west of and within the Dahomey Gap for our data set: sample sizes are low for pre-1960, 1980–1989, 1990–2000, and 2010–present (Fig. 2B; Supplemental Material S1). Most sampling took place between 1960 and 1969, 1970 and 1979, and 2000 and 2009. These trends are also reflected when we look at samples collected in Ghana alone, indicating that sampling in Ghana represents a large proportion of all samples collected in West Africa (Fig. 2B). The number of *Bd*-positive samples in Africa is low until the turn of the century, and increases in 2010–present, despite a decrease in sample size (Fig. 2B). In West Africa west of and within the Dahomey Gap, only 1960–1969 and 1970–1979 had *Bd*-positive samples, which were detected in the present study.

Phylogenetic Analysis of West African *Bd*

One of five *Bd*-positive samples produced enough sequence data to be included in our *Bd* phylogenetic analysis (CAS 153500; $n = 7$ amplicon sequences). The consensus tree had a

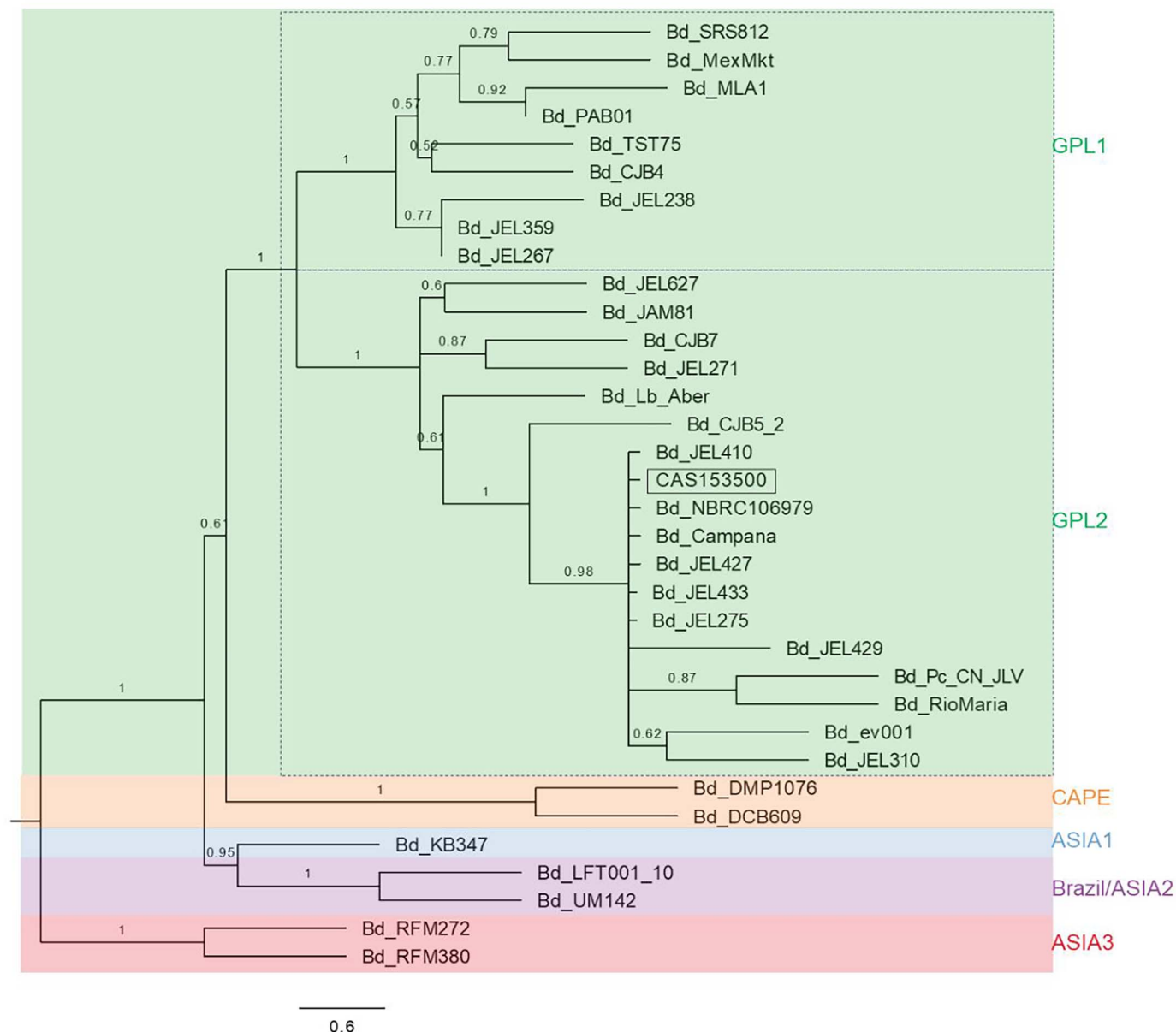


FIG. 3.—*Batrachochytrium dendrobatidis* (Bd) lineage in West Africa. Consensus tree calculated from 92 amplicon trees showing that sample CAS 153500 belongs to global panzootic lineage of *Bd* (Bd-GPL) and is within the *Bd*-GPL2 sublineage. Nodes are labeled with posterior probability and nodes with a posterior probability <0.5 have been collapsed. Major *Bd* lineages are labeled and colored as in Byrne et al. (2019).

normalized quartet score of 0.82 (or proportion of amplicon trees satisfied by the consensus tree) and included all known lineages of *Bd* (Fig. 3). In our consensus tree, CAS 153500 was nested within the global panzootic lineage of *Bd* (*Bd*-GPL) and, more specifically, belongs to the *Bd*-GPL2 clade (Fig. 3). Another sample (CAS 153502) returned only a single amplicon sequence; however, by analyzing the sequence alignment for this amplicon (AB176) we see that this sequence is identical to all other *Bd*-GPL sequences, including CAS 153500 (Fig. 4), meaning this sample is likely also within the *Bd*-GPL lineages.

DISCUSSION

We show that *Bd* has been present in West Africa for at least 54 yr but at low prevalence, which is not unusual for

studies screening museum specimens. Our specimens date back as far as 1912; therefore *Bd* zoospores have likely degraded over time, resulting in low zoospore yields and increased probability of false negatives (Talley et al. 2015; Hirschfeld et al. 2016; Hydeman et al. 2017). The potential for detecting false positives from cross-contamination is also a possibility in any *Bd* screening study. Regardless, the number of *Bd*-positive samples is typically an underestimation of true *Bd* prevalence when working with historical specimens. Our estimates of *Bd* prevalence are consistent with studies that screened for *Bd* using museum collections from other African regions (2.7% [19/697], Weldon et al. 2004; 1% [6/620], Soto-Azat et al. 2010). When compared with museum studies that sampled amphibians from North America (13.1% prevalence, Ouellet et al. 2005; 11.1%, Talley et al. 2015), Mexico (25.5–27.7%, Basanta et al. 2021), and Italy



FIG. 4.—Sequence alignment for amplicon AB176 showing that both CAS 153500 and CAS 153502 sequences are identical to all other *Batrachochytrium dendrobatidis* (*Bd*)-GPL sequences and are distinguishable from the other *Bd* lineages. *Bd*-lineage groupings are labeled as in Fig. 3. IUPAC ambiguity codes are used for samples with multiple alleles.

(62.5%, Canestrelli et al. 2013), however, historical *Bd* prevalence in West Africa is relatively low. Low *Bd* prevalence, along with no detection of *Bd* in our more recent museum samples (year 2000 or later; $n = 1000$), could suggest that *Bd* is not a major causative agent of amphibian declines in West Africa. However, additional testing of museum and contemporary samples is warranted to give a better understanding of *Bd* prevalence through space and time in this region.

Of interest, all the *Bd*-positive samples were of a single species, *K. senegalensis* (Family: Hyperoliidae; 5/56 infected; 95% CI = $0.03 \leq 0.09 \leq 0.20$). This species is an aquatic breeder, widely distributed across the tropical savanna of Africa, and occupies a diverse range of habitats (shrublands, grasslands, wetlands, montane forests, degraded forests; Lanza 1981). Previous studies detected *Bd* in *K. senegalensis* from Udzungwa Mountains, Tanzania at high prevalence (5/7 infected; 71.43%; Moyer and Weldon 2006) and *Kassina* sp. in South Africa (6/28 infected; 21.4%; Tarrant 2012). Therefore, targeted sampling of *K. senegalensis* could reveal that this species is particularly susceptible to *Bd* infection compared with other widespread West African species or whether it may be a vector of *Bd* across otherwise inhospitable terrain.

Four of the *K. senegalensis* samples were collected at the University of Ghana campus, which is an urbanized site in Accra receiving substantial foot traffic. It has been hypothesized that Accra is the most likely entry point for *Bd* given extensive road networks that allow direct entry into the capital from surrounding countries (Penner et al. 2013), facilitating pet and wildlife trade. Therefore, transport of amphibians into and around Ghana could be a factor explaining the positive occurrences in Accra since international trade is linked to the spread and introduction of *Bd* to naïve host communities around the world (Daszak et al. 2004; Weldon et al. 2004; Fisher et al. 2009; Kolby et al. 2014; Wombwell et al. 2016).

Conversely, given the *Bd*-positive sample in Benin, an area previously hypothesized to serve as a geographical barrier to *Bd* dispersal (Penner et al. 2013), it is also possible that *Bd* naturally dispersed northward into Ghana from Nigeria and neighboring Central African countries. The positive occurrence in Benin suggests that the Dahomey Gap is hospitable for *Bd*, and therefore *Bd* could spread into countries west of the Dahomey Gap from neighboring countries with high prevalence such as Nigeria and Cameroon (Zimkus et al. 2020). Our phylogenetic analysis of the *Bd*-positive sample collected from Ghana indicates the lineage is *Bd*-GPL2, which differs from the dominant lineage in Cameroon (*Bd*-CAPE; O'Hanlon et al. 2018) and therefore may suggest an alternate origin of *Bd* in West Africa. This *Bd* genotype is associated with massive amphibian die-offs in the Neotropics and Australia (Schloegel et al. 2012; James et al. 2015; Basanta et al. 2021). The other sample for which we recovered a single amplicon also matched the sequence for *Bd*-GPL2, but whether *Bd* was endemic or introduced, the strains that may be present in contemporary samples remain unclear. Despite the discovery of *Bd*-GPL in West Africa and other regions in Africa such as Burundi, Kenya, Ethiopia, Madagascar, South Africa, and the Gulf of Guinea Islands (Byrne et al. 2019), no significant *Bd*-driven amphibian declines have been recorded in these areas.

Our results also highlight the critical relationship between sampling effort and detection probability. For instance, biological surveys of amphibians in West Africa are disproportionately concentrated in Ghana, underscoring geographic biases in sampling. There are 9221 West African specimens in databased collections, 4013 (44%) of which are specimens from Ghana. In the present study, 2289 (74%) of the 3093 specimens screened were collected from Ghana. Similarly, Penner et al. (2013) surveyed 793 amphibians, 290 (37%) of which were collected from Ghana. Comparing Clopper–Pearson CIs for Ghana specimens in the present study (95% CI = $0.0005 \leq 0.0017 \leq 0.0045$) with those in Penner et al.

(2013; 95% CI = 0.000–0.013) demonstrates that large sample sizes are necessary to detect *Bd* when prevalence is low: we sampled four times as many amphibians and detected only four *Bd*-positive occurrences from Ghana. This difference in sampling effort may offer a partial explanation as to why Penner et al. (2013) did not detect *Bd* in West Africa. Furthermore, our study demonstrates how leveraging museum collections can inform amphibian conservation efforts in West Africa. Because of the dearth of amphibian surveys and formerly considered absence of *Bd* in West Africa, *Bd* management strategies have yet to be established for this region. Management strategies should include increased regulation of international amphibian trade since it is implicated in the emergence and spread of *Bd*, with at least 28 species of introduced amphibians also being known carriers of *Bd* (Fisher and Garner 2007). Thus, anthropogenically mediated spread of *Bd* is a major concern, particularly in areas with high species diversity such as West Africa (Penner et al. 2011) and because environmental factors can exacerbate the effects of *Bd* infection on African amphibian assemblages. For example, habitat fragmentation and climate change are major threats to amphibian species diversity in West Africa. Since the 1960s, West African rainforests—the principal habitat of amphibians in this region—have decreased in area by >80% (Chatelain et al. 1996). Moreover, host-species range and *Bd* habitat suitability are likely to shift because of climate change (Xie et al. 2016). Threats to amphibian diversity are additive; therefore, habitat fragmentation and climate change may increase the severity of *Bd*-driven declines (Xie et al. 2016). These anthropogenic threats may act synergistically with *Bd* to drive amphibian declines, which can result in devastating, cascading effects on ecosystem function and community structure (Colón-Gaud et al. 2009).

In conclusion, we show that the *Bd* fungus has been in West Africa since at least 1969 but whether the pathogen is present there today remains uncertain. Future *Bd* surveys should target Benin and Ghana (where *Bd* was detected in the present study) along with other parts of the Dahomey Gap. Our study challenges the present characterization of West Africa as a “safe haven” for amphibians and establishes groundwork for future *Bd* studies and amphibian conservation efforts in this region.

SUPPLEMENTAL MATERIAL

Supplemental material associated with this article can be found online at <https://doi.org/10.1655/Herpetologica-D-24-00049.S1>; <https://doi.org/10.1655/Herpetologica-D-24-00049.S2>.

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